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First record of troglobitic Bothriuridae (Scorpiones) in Australia from the Pilbara

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Abstract

We report the first troglobitic members of the scorpion family Bothriuridae from Australia, discovered in the Pilbara region of Western Australia, and provide a brief review of Australian troglofaunal scorpions. Scorpions are rare in subterranean ecosystems, with only 28 species recorded globally. Two described and two undescribed subterranean scorpion species were previously known from Australia. The Pilbara specimen documented herein exhibit pronounced troglomorphic traits, including complete loss of ocelli and pigmentation and weakened/diminished appendages, consistent with obligate subterranean adaptation. Morphological and phylogenetic analyses place the specimen within Bothriuridae. Photographs, collection details, and molecular data are provided. Together with a previously collected undescribed bothriurid from the Pilbara, this discovery represents the first documented troglobitic Bothriuridae globally. Formal taxonomic treatment will follow in a subsequent dedicated study.

Keywords

Australia, Bothriuridae, Pilbara, scorpion, subterranean fauna, troglobitic, troglomorphy

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Introduction

Western Australia (WA) harbors a staggering subterranean faunal diversity (Guzik et al. 2011; Majer et al. 2018). Most subterranean specimens are collected as part of environmental impact assessments for potential resource developments. These surveys target invertebrates living in underground interstices and cavities between the water table and the superficial soil layer (the vadose zone; troglofauna) or within groundwater (stygofauna) (Humphreys et al. 2013). Unlike the large karst systems known globally to host diverse subterranean communities, underground habitats in WA mostly comprise vugs and voids, also referred to as meso- and micro-caverns, and are typically inhabited by small invertebrates such as slaters, millipedes, cockroaches, centipedes and spiders. Larger animals, including scorpions and vertebrates, appear to be extremely uncommon in these systems, likely because the size and connectivity of voids limit their persistence underground.

Against this backdrop, the rarity of scorpions in subterranean environments is striking. Scorpions are a common component of the terrestrial fauna in arid Western Australia, yet very few have been recorded living underground. In Australia, the group is dominated by two families—Urodacidae and Buthidae—with two additional families (Hormuridae and Bothriuridae) occurring primarily in cooler and wetter (mesic) regions. Urodacidae is represented by two genera, *Aops* (Volschenk and Prendini 2008) and *Urodacus* (Peters, 1861), with *Aops* being represented by a single species. The family Buthidae is comprised of five genera, *Australobuthus* (Lockett, 1990; SA salt lakes), *Lychas* (Koch, 1845; Australia-wide), *Hemilychas* (Hirst, 1911; Australia-wide), *Isometroides* (Keyserling, 1885; Australia-wide) and *Isometrus* (Ehrenberg, 1828; eastern and northern Australia). The family Hormuridae is comprised of two genera, *Hormurus* (Thorell, 1876) and *Liocheles* (Sundevall, 1833), which inhabit warm to cool and wet climates in the far northern Kimberley and Northern Territory, as well as the wet rainforest along the Queensland coast. Bothriuridae is represented by a single Australian endemic genus, *Cercophonius* (Peters, 1861). The genus is represented by six described species that mostly inhabit the mesic climates of south-west of WA, southern South Australia, Victoria, coastal New South Wales and Tasmania.

There have only been four subterranean scorpion species collected in Australia – two described and two undescribed (Fig. 1). The first subterranean scorpion discovered was *Hormorus polisorum* (Volschenk et al. 2001; ex *Liocheles*, family Hormuridae) from two karst caves (Bishops Cave CI-8 and 19th Cave CI-19) underneath Christmas Island (Humphreys and Eberhard 2001; Volschenk et al. 2001). *H. polisorum* exhibits partial to complete subterranean adaptation and is classified as a troglobite (Prendini et al. 2021). The second species is *Aops oncodactylus* (Volschenk & Prendini, 2008; Urodacidae), a troglobite from the limestone caves underneath Barrow Island, which is located 50 km northwest of the Pilbara coast. The third is from the family Buthidae (possibly in the genus *Lychas*), collected from the Judbarra/Gregory Karst Area, Northern Territory and lacks formal description (Moulds and Bannink 2012). The *Lychas*? specimen was collected from deep within the cave system and based on a brief examination by the authors, exhibits partial reduction of pigment, developed ocelli and

Figure 1. A troglofaunal scorpions recorded from Australia **B** inset of two troglobitic Bothriuridae from the Pilbara **C** example of a mineral exploration borehole and modified plankton net used to collect the Bothriuridae specimens. The visible PVC casing extends several metres below ground, isolating the soil profile and stabilising the bore entrance; sampling was conducted from deeper meso- and micro-cavernous strata.

the legs are elongated (Moulds and Bannink 2012); this individual could be classed as a troglophile. The fourth is an undescribed scorpion collected in the Hamersley Range, Pilbara, Western Australia, during surveys of the Flinders Pilbara Iron Ore Project (Worley Parsons 2012). The specimen is currently held at the WA Museum (Registration Number T127999) and belongs to the family Bothriuridae (WAM 2022). It was collected in 2012, and based on a brief examination by the authors, it lacks ocelli and pigmentation and is a troglobite. Multiple attempts at genetic sequencing of specimen T127999 by the WA Museum have been unsuccessful (M. Harvey pers. comm.).

In this paper we present a brief description of the morphology, ecology, genetics, and subterranean habitat of a troglobitic scorpion collected in the Pilbara region of WA and outline future steps for its taxonomic treatment. Assessments of troglomorphy follow the ecological classification of cavernicolous and troglomorphic scorpions in Prendini et al. (2021).

Methods

Field and morphology

The specimen was collected from an uncased vertical mineral exploration hole (RC06BS3M019) located at 543776.6 mE 7508202.0 mN (GDA94 MGA Zone 50; 22°31'55.91"S, 117°25'32.56"E) on 23 February 2021 (Fig. 1). The bore was scraped from 0 to 36 m below ground using a modified plankton net by Syngeon Rodman and Joel Huey. The net was lowered to the bottom of the hole and raised four times, after which the contents were elutriated, washed, and preserved in chilled 95% ethanol. The sample was stored at –4 °C for four days before being transferred to the Biologic laboratory, where it was sorted and stored in refreshed 95% ethanol at –20 °C.

The specimen is registered with the Western Australian Museum (WAM T162723). GenBank accession numbers are COI: OR252705, 16S PZ102470, and 28S PZ102471.

The stratigraphy of hole RC06BS3M019 comprises 0–22 m pisolithic detritals, 22–34 m calcrete, and 34–36 m West Angelas Shale (Wittenoom Formation). The pisolithic detritals and calcrete contain pore spaces suitable for troglofauna, whereas the underlying West Angelas Shale does not.

The specimen was photographed under a Leica M210 stereoscope using LED and ultraviolet illumination. Morphological examination followed the keys of Prendini (2000).

Molecular

Specimen extraction and amplification

Genomic DNA was extracted from a portion of tissue removed from the second left leg of the specimen using the Qiagen DNeasy tissue and blood-extraction kit (QIAGEN, Venlo, The Netherlands), following the manufacturer's instructions. DNA was eluted into 25 µL of buffer and stored at 4 °C until required for further analysis. Three genes were chosen to amplify in the specimen: cytochrome c oxidase subunit I (COI), 16S rRNA (16S) and 28S rRNA (28S). Primers are shown in Table 1 (Folmer et al. 1994; Nunn et al. 1996; Palumbi 1996; Simon et al. 1994; Whiting et al. 1997).

Table 1. Primers used in this study (Folmer et al. 1994; Nunn et al. 1996; Palumbi 1996; Simon et al. 1994; Whiting et al. 1997).

Gene	Prime Name	Prime Sequence (5'-3')
COI	LCO1490	GGTCAACAAATCATAAAGATATTGG
	HC02198	CTAAACTTCAGGGTGACCAAAAAATCA
16S	16Sar	CGCCTGTTTATCAAAAACAT
	16Sb	CTCCGGTTTGAACTCAGATCA
28S	28Sa	GACCCGTCTTGAAACACGGA
	28Sb	TCGGAAGGAACCAGCTAC

Polymerase chain reactions (PCRs) were performed in 25 µL reactions, which included 2 µL of template DNA, 0.2 µL of each primer, 1 unit of taq Bioline Redmix (Bioline, London, UK). COI and 16S PCR reactions were run using the following protocol: 95 °C for 5 min; then 35 cycles of 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 45 s; with a final extension at 72 °C for 10 min. The 28S PCR reaction was run using the following protocol: 95 °C for 3 min; then 35 cycles of 95 °C for 30 s, 49 °C for 30 s, and 72 °C for 45 s; with a final extension at 72 °C for 10 min. PCR products were visualised on E-Gel® pre-cast agarose gels (Life Technologies, Melbourne, Vic, Australia). Bi-directional sequencing was performed at the Australian Genome Research Facility (AGRF), and sequences and workflows were managed in the Geneious Prime software package (version 2022.2.1; Kearse et al. 2012), using the LIMS Biocode plug-in (http://www.mooreabiocode.org). Sequence editing occurred in Geneious Prime, and the COI sequence was checked for contamination by translating sequences to amino acids to search for internal stop codons. The resulting sequences were "BLASTed" against the GenBank nucleotide collection using "blastn" returning 50 hits to test for contamination, and to confirm that the putative morphological identification was supported by each gene independently.

Phylogenetic analysis

A comparative dataset was constructed from sequences available on GenBank. Exemplar bothriurids and other scorpion families were selected from GenBank, including those from Ojanguren-Affilastro et al. (2016), which included a representative of the only Australian bothriurid genus, *Cercophonius* (Table 2). Datasets were aligned using the MAFFT plugin in Geneious (Katoh and Standley 2013). MAFFT was run with the 'Auto' algorithm, with 200PAM/k = 2 scoring matrix, open-gap penalty of 1.53, and an offset value of 0.123.

Individual gene trees were built using the RaxML plugin in Geneious (Stamatakis 2006), with the GTR+GAMMA rate heterogeneity model with rapid bootstrapping and 1,000 bootstraps. A concatenated gene tree was constructed and analysed with RaxML and the same parameters, partitioned by gene, and COI partitioned by codon position.

Table 2. Comparative dataset of exemplar Bothriurids and other scorpion families from GenBank used in phylogenetic analysis.

Taxon name	Family	Voucher	COI	16S	28S
Belisarius xambeui	Belisariidae	MNHN-JAD29	JN018156		JN018370
Bothriuridae sp. Biologic-SCOR002	Bothriuridae	WAMT162723/ BES11137_BMR02659	OR252705	PZ102470	PZ102471
Bothriurus flavidus	Bothriuridae	LP_3452	KT447034	KT446699	KT446923
Brachistosternus angustimanus	Bothriuridae	LP_2254	KT446934	KT446597	KT446825
Brachistosternus paposo	Bothriuridae	LP_11301/S058	KX517244	KT446655	KT446879
Brachistosternus roigalsinai	Bothriuridae	LP_7627/S050	KX517238	KT446685	KT446909
Brachistosternus sp. 1 LP-2016	Bothriuridae	AMCC:LP8343	KU755644	KU755580	KU755623
Brachistosternus sp. 8 LP-2016	Bothriuridae	AMCC:LP10650	KU755653	KU755590	KU755632
Brachistosternus telteca	Bothriuridae	LP_2380	KT447024	KT446689	KT446913
Cercophonius sulcatus	Bothriuridae	LP_1618	KT447038	KT446703	KT446927
Ananteris ashaninka	Buthidae	S996	ON255558		ON259166
Ananteris franckei	Buthidae	SC01			KP276375
Megacormus gertschi	Euscorpiidae	46scrp/S1619	MW250330	MF134688	MF134751
Liocheles australasiae	Hormuridae	Sc638/MZMU2135	MW387531	JQ514233	KY271345
Heterometrus laoticus	Scorpionidae	MNHN-JAB36	AY156573	AY156558	JN018374
Pandinus imperator	Scorpionidae	AMCC 101703	AY156582	AY156567	AY156537
Urodacus novaehollandiae	Urodacidae	MCZ IZ-46897			KY573697
Kochius magdalensis	Vaejovidae	LP8796	KM274772	KM274334	KM274626
Mesomexovis aff. *punctatus*	Vaejovidae	34scrp		MF134682	MF134745
Mesomexovis aff. *punctatus*	Vaejovidae	20scrp	MF134786		
Mesomexovis aff. *subcristatus*	Vaejovidae	36scrp	MF134781	MF134680	MF134748
Smeringurus vachoni	Vaejovidae	DMNS29872/S1569	MW250329	MF061499	
Vizcaino viscainensis	Vaejovidae	LP3174	KM274864	KM274426	KM274718

Results

Morphology

The specimen exhibits clear troglomorphic features consistent with an obligate troglobite, including complete absence of eyes and pigmentation and pronounced weakening/diminished appendages, namely the pedipalp. The full body length is 13.7 mm, measured from the posterior tip of the telson to the anterior edge of the prosoma. The sternum is subpentagonal with posterior emargination and well-developed lateral lobes connected medially, corresponding to sternum form "external view type 2" of Soleglad and Fet (2003). The coxapophysis is anteriorly blunt and not pointed. These characters, together with appendage diminishment/weakening and vesicle enlargement, support classification as a hypogean troglobite under the framework of Prendini et al. (2021). Specimen photographs are provided in Fig. 2, followed by a photo of the sternal plate in Fig. 3.

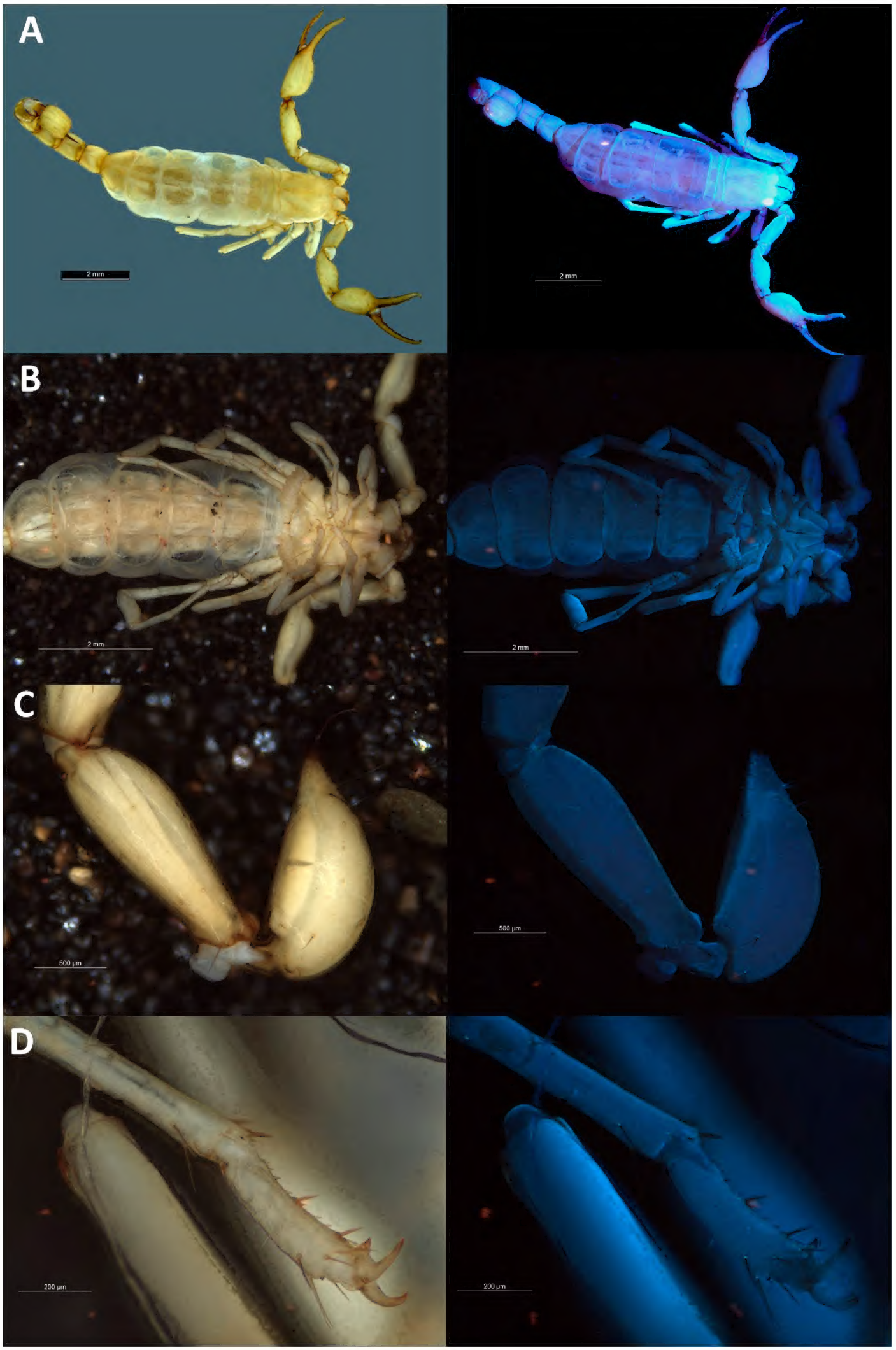


Figure 2. Bothriuridae sp. Biologic-SCOR002 in white and UV light **A** Dorsal aspect **B** Mesasoma, ventral aspect **C** Metasoma telson, lateral aspect **D** Telotarsus of leg IV **E** Carapace **F** Chelicerae **G** Left pedipalp patella, dorsal aspect **H** Right pedipalp chela.

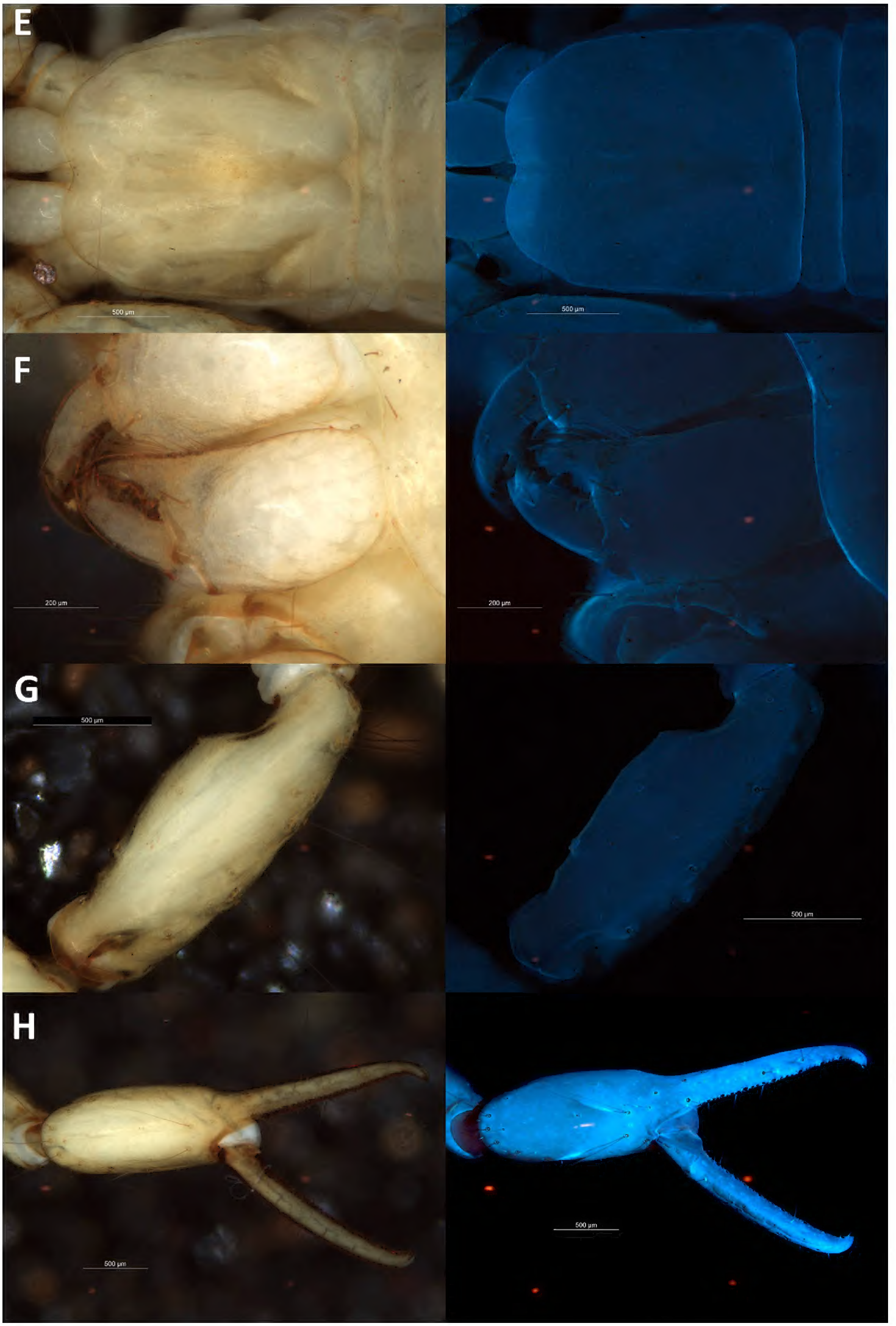
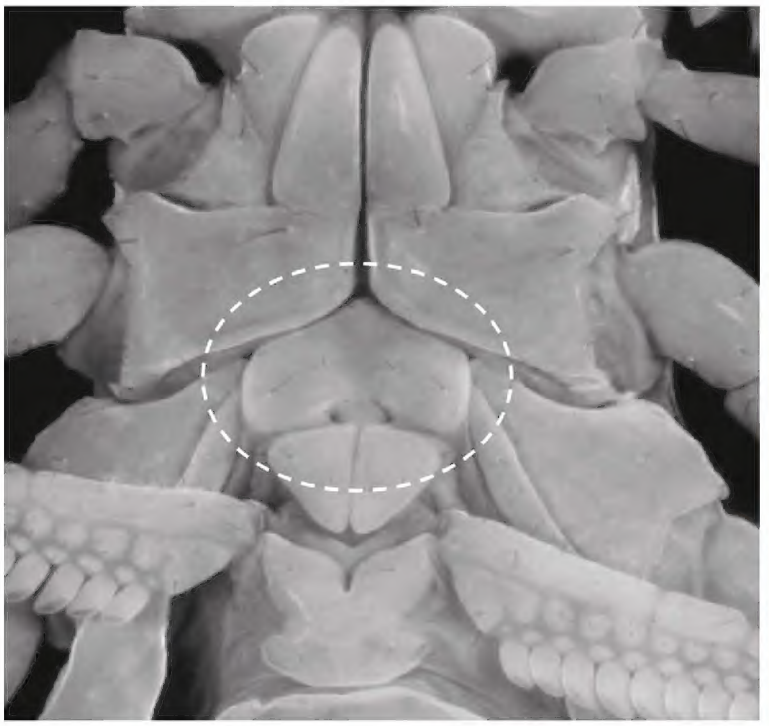
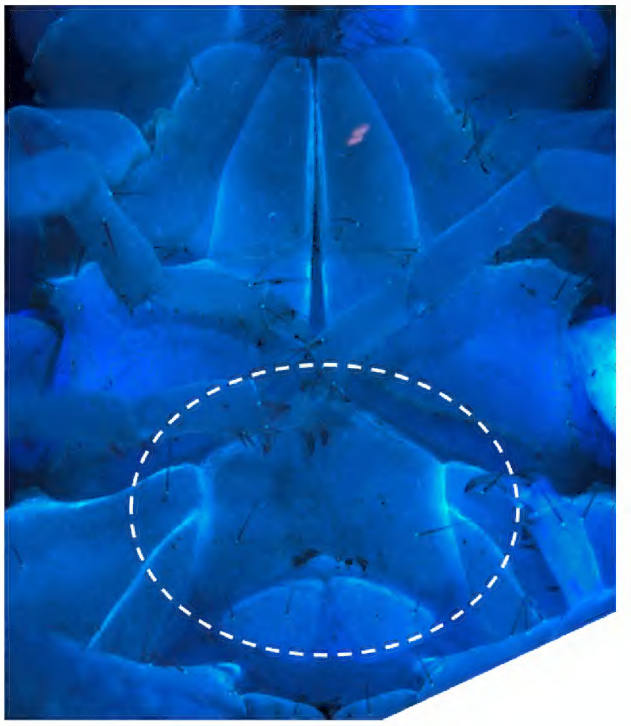


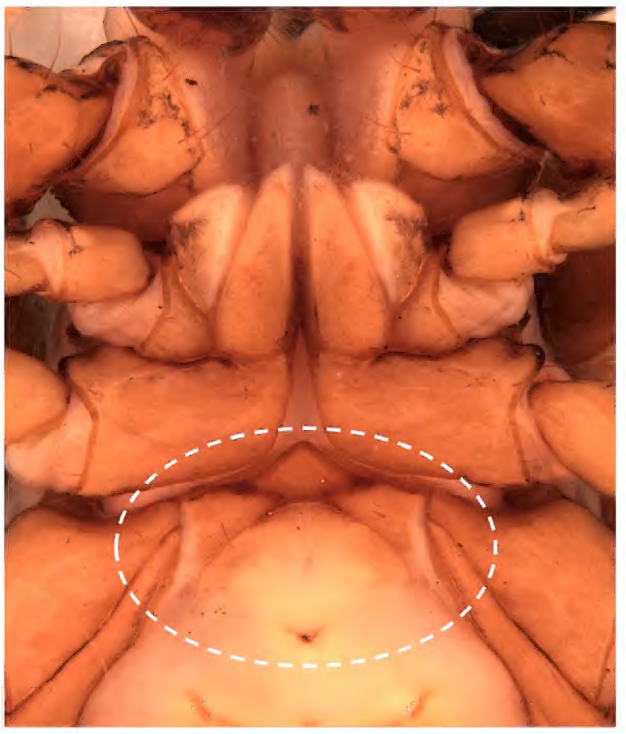
Figure 2. Continued.



Mauryius Ojanguren-Affilastro, 2017
South America



Blind scorpion
Pilbara, Site RC06BS3M019



Cercophonius sulcatus (fem)
WAM T90-1114, SW WA

Cercophonius sulcatus (juv)
WAM T97-1743, SW WA

Figure 3. Sternal plates of *Mauryius* (Ojanguren-Affilastro and Mattoni 2017), Bothriuridae sp. Biologic-SCOR002, and a female and juvenile representative of *Cercophonius sulcatus* (WA Museum T90-1114, T97-1743).

Habitat

The specimen was recovered from meso- or micro-cavernous voids between 0 and 34 m below surface within pisolithic detritals and calcrete.

Molecular results

Overall, molecular analyses consistently supported placement of the target specimen within Bothriuridae. All three gene fragments were successfully amplified, sequenced, and passed contamination checks, yielding fragments of 454 bp (COI), 528 bp (16S), and 354 bp (28S). BLAST searches for each gene returned members of Bothriuridae as the closest matches.

For 16S, the top two hits (ranked by bit score) were *Cercophonius sulcatus* (Kraepelin, 1908; KT446703) and *Urophonius brachycentrus* (Thorell, 1876; KT446702), followed by *Uroctonus mordax* (Thorell, 1876; Chactidae; EU523756). In total, 46 of the top 50 hits were bothriurids. For COI, the top matches were *Bothriurus flavidus* (Kraepelin, 1911; KT447034), *Brachistosternus ferrugineus* (Thorell, 1876; KT446960, KT446962), and *Urophonius brachycentrus* (Thorell, 1876; KT447037), followed by *Heterometrus cyaneus* (Ehrenberg, 1828; Scorpionidae; MT418094); 16 of the top 50 hits were bothriurids. For 28S, all 50 top hits were bothriurids, with *Bothriurus* sp. (Koch, 1842; KP276379), *Cercophonius sulcatus* (KT446927), and *Bothriurus flavidus* (KT446923) ranking highest.

Individual gene trees consistently recovered the target specimen as sister to *Cercophonius sulcatus*, the only Australian representative of Bothriuridae included in the analysis. Bootstrap support for this relationship was low to moderate across genes (COI bs = 75; 28S bs = 35; 16S bs = 63). The pairwise COI distance between the two taxa was 16.52%. The concatenated tree recovered the target specimen as sister to *Cercophonius sulcatus* with stronger support (bootstrap = 97). It was also well nested within Bothriuridae. A summarised phylogeny is presented in Fig. 4, in which the specimen is designated as Bothriuridae sp. Biologic-SCOR002.

Discussion

These discoveries demonstrate that even comparatively well-surveyed subterranean systems may harbour large-bodied, highly troglomorphic taxa that remain undetected due to ecological and methodological biases. Bothriuridae sp. Biologic-SCOR002 and WAM specimen T127999 represent the first documented troglobitic scorpions recorded from the Pilbara region of Western Australia and the first documented confirmed troglobitic members of Bothriuridae globally. This increases the recognised number of troglobitic scorpions from 28 to 30 (Prendini et al. 2021). We note that the troglomorphism of the *Lychas?* specimen from the Northern Territory (Moulds and Bannink 2012) warrants reassessment to determine whether it should also be included in this count.

As of 2019, approximately 3,500 subterranean fauna samples (from mineral exploration holes) have been collected from the Brockman Syncline/Jeerinah Anticline system (Biologic 2018, 2022a) and approximately 50,000 from the Pilbara region more broadly (WABSI 2021). Despite this extensive sampling effort, only two troglobitic scorpions have been documented. Larger animals such as scorpions may be less

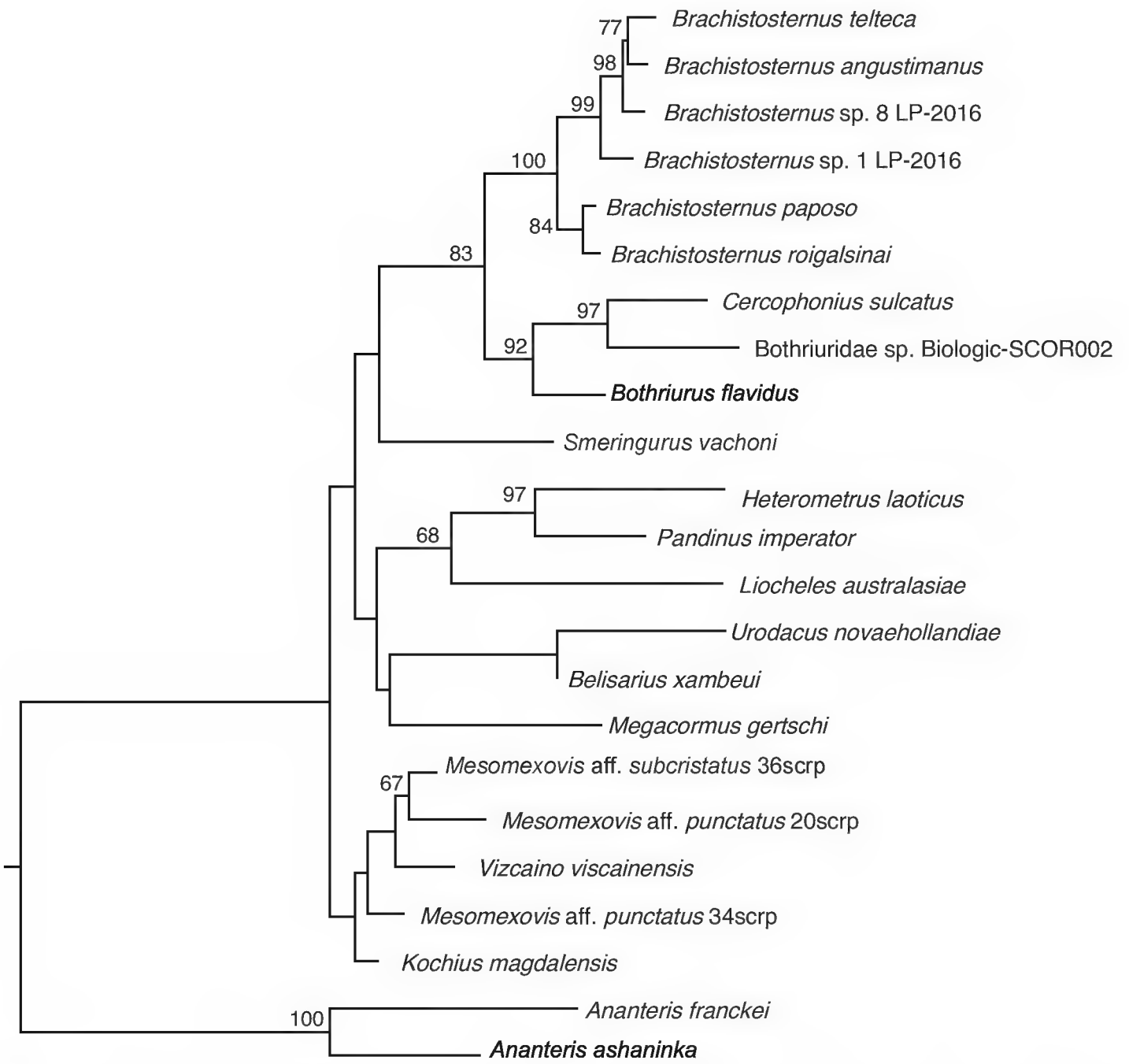


Figure 4. Concatenated phylogenetic tree including the target specimen Bothriuridae sp. Biologic-SCOR002 and its most closely related species. The family Bothriuridae is indicated in the grey dashed box.

successful in colonising and persisting in the Pilbara's subterranean environments due to limited habitat connectivity and resource availability. Moreover, Bothriuridae as a family would not be considered an obvious candidate for subterranean specialisation. Globally, most bothriurids are fossorial, with some lithophilous and arboreal species (Pizarro-Araya et al. 2011; Ojanguren-Affilastro et al. 2012; Ojanguren-Affilastro and Mattoni 2017), and no troglobitic representatives are known from the family's current epicentres of diversity in Andean and Brazilian cave systems. In addition, borehole sampling methods likely bias against detecting larger troglobitic taxa. The present discovery therefore underscores both the rarity of such taxa and the limitations of current survey approaches.

In the Pilbara region of Western Australia, obligate troglobitic fauna commonly occupy meso- and micro-cavernous habitats within calcrete, detritals, and fractured rock rather than human-accessible caves. Accordingly, classification as troglobitic does

not require collection from a cave environment but is inferred from morphology, ecology, and consistency with established frameworks (Prendini et al. 2021). The collection site lies within the Brockman Syncline (Butt et al. 2001), where geomorphology is structured by the erosional resistance of the Brockman and Marra Mamba iron formations. The specimen was recovered from pisolithic detritals and calcrete, units that are highly prospective for troglofauna and likely provide suitable structural conditions for obligate subterranean taxa. These cavernous detrital systems, underlain by relatively impermeable West Angelas Shale, may provide the structural stability necessary for long-term persistence of larger hypogean species.

Specimen T127999 was recorded from the Hamersley Range, approximately 48 km north of the present collection site. These two records are separated geologically by Caves Creek, a major drainage channel. Based on preliminary morphological comparison, differences between the specimens are apparent, although formal morphological work is required. Genetic sequencing of T127999 has been unsuccessful and therefore it could not be included in comparative molecular analyses. Given the geographic separation and intervening geology, the two specimens likely represent distinct species. Sequencing of additional troglobitic taxa from both locations would help clarify patterns of gene flow and colonisation.

Although spatially disjunct, both records occur within iron-rich detrital systems of the central Pilbara, suggesting that this geological setting may be particularly favourable for troglofauna. Subterranean fauna surveys of the Brockman Syncline and the Flinders Pilbara Iron Ore Project recorded numerous higher-order troglofaunal groups, yet only these two troglobitic scorpions have been documented. The geology at Flinders comprises outcropping banded iron formation (BIF) and channel iron deposits formed through cyclic weathering and erosion, further indicating that cavernous iron-rich detrital systems can provide suitable structural conditions for persistence of larger obligate subterranean taxa.

Biogeography

The occurrence of two obligate troglobitic bothriurids in the Pilbara is exceptional in both an Australian and global context. The family Bothriuridae is a temperate Gondwanan lineage within the superfamily Bothriuroidea, with origins dating to the Permian or earlier (Sharma et al. 2018). In Australia, bothriurids are largely restricted to the southern mesic fringes of the continent, a pattern typical of Gondwanan taxa (Rix et al. 2015). This distribution reflects the contraction and fragmentation of mesic biota following aridification of the continent approximately 20 million years ago (Byrne et al. 2011, 2008). Taxa adapted to widespread mesic forests persisted in refugia, including southern coastal regions, topographically buffered habitats, and subterranean environments.

It is plausible that the two Pilbara subterranean scorpions represent climatic relicts, sharing ancestry with mesic-restricted *Cercophonius* genus prior to the onset of aridification. The ~16% COI divergence between Bothriuridae sp. Biologic-SCOR002 and *Cercophonius sulcatus* is consistent with substantial evolutionary separation. Applying

an approximate divergence rate of 1.4% per million years (derived from Buthidae; Gantenbein et al. 2005) yields an estimated divergence time of approximately 11–12 million years, consistent with the progressive aridification of the Australian continent (Byrne et al. 2011, 2008). The sternum morphology, which shares similarities with that of juvenile *C. sulcatus* (Fig. 3), is compatible with placement Bothriuridae sp. Biologic-SCOR002 within *Cercophonius*, as a distinct but related lineage.

A second morphological interpretation suggests this taxon lies outside *Cercophonius*. The non-transverse subpentagonal sternum of Bothriuridae sp. Biologic-SCOR002, a character otherwise observed in South American genera (*Tehuankea* Cekalovic, 1973, *Mauryius* Ojanguren-Affilastro & Mattoni, 2017) and African genera (*Lisposoma* Lawrence, 1928, *Brandbergia* Prendini 2008), raises the possibility that this taxon represents a distinct lineage within the family. If so, divergence may predate Australian aridification and reflect deeper Gondwanan history. This interpretation is consistent with dated phylogenomic analyses of Bothriuridae based on UCEs and transcriptomic datasets, which suggest a deep (~94 Ma) split between South American and Australian bothriurids, consistent with dispersal through temperate Gondwana prior to final breakup of the South America-Antarctica-Australia connection (Ojanguren-Affilastro et al. 2023). A more comprehensive molecular clock analysis with broader taxon sampling would be required to test either hypothesis robustly.

Conservation and further studies

The Pilbara region hosts significant mineral resources and is a global hotspot for subterranean biodiversity. Most subterranean fauna surveys are conducted to inform environmental impact assessments (EPA 2021). Increased molecular sequencing over the past decade has revealed considerable heterogeneity in rates of speciation and modes of subterranean colonisation across taxa.

Despite extensive mineral exploration drilling that provides access to the vadose zone, substantial challenges remain in assessing the true geographic extent of obligate troglofauna. Drill holes are spatially constrained, may not intersect suitable voids, and are often rehabilitated or collapse due to geological instability. Low capture rates and uneven uptake of molecular sequencing further limit inference on distribution and connectivity.

Obligate troglofauna in the Pilbara are typically short-range endemics (Abrams et al. 2020; Harvey et al. 2011). Bothriuridae sp. Biologic-SCOR002 and Bothriuridae sp. T127999 are currently known from single sites and, consistent with regional patterns, should provisionally be regarded as short-range endemics pending further data. While singleton status in the Pilbara often reflects sampling limitations rather than confirmed rarity, the relatively large body size and likely higher trophic position of these scorpions suggest that they may represent ecologically significant components of subterranean food webs. The potential ranges of these species could be derived via predictive three-dimensional habitat modelling, which is currently utilised in locations of resource mineral exploration drilling (see Biologic 2022b), coupled with targeted sampling approaches such as wet pit traps deployed down mineral exploration holes and environmental DNA

(Saccò et al. 2022). Formal taxonomic resolution remains a priority for understanding their distribution, evolutionary history, and conservation status.

Together, these findings underscore the evolutionary and conservation significance of subterranean systems in the Pilbara and highlight the need for integrative taxonomic and ecological investigation of Australia's hypogean fauna.

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